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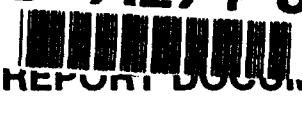

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13. ABSTRACT (MAX 200 WORDS): THE K+ CHANNEL OPENERS CROMAKALIM AND PINACIDIL INDUCED A CONCENTRATION-DEPENDENT REDUCTION IN ATRIAL CONTRACTION FORCE WITH EC50 VALUES OF 25+- 2 AND 37+- 2 UMOL/L, RESPECTIVELY. THIS DEPRESSANT EFFECT WAS ANTAGONISED BY 50 UMOL/L TACRINE WHICH DISPLACED THE CONCENTRATION-RESPONSE CURVES OF CROMAKALIM AND PINACIDIL TO THE RIGHT. THE RESPECTIVE DR50 VALUES WERE 3.8 AND 2.3. INCREASING THE TACRINE CONCENTRATION (100 AND 500 UMOL/L) PRODUCED NO ADDITIONAL EFFECT ON THE CONCENTRATION-RESPONSE RELATIONSHIPS. ADDITION OF 1 UMOL/L ATROPINE ENHANCED THE ANTAGONISM DUE TO TACRINE BY INCREASING THE DR VALUE FROM 3.8 TO 6.5 FOR CROMALALIM AND FROM 2.3 TO 5.2 FOR PINACIDIL. GLIBENCLAMIDE, AN ATP-SENSITIVE K+ CHANNEL BLOCKER, COMPETITIVELY INHIBITED THE NEGATIVE INOTROPIC EFFECTS OF CROMAKALIM AND PINACIDIL. THE RESPECTIVE DISOCIATION CONSTANTS FOR GLIBENCLAMIDE AGAINST CROMAKALIM AND PINACIDIL WERE 0.57 AND 0.35 UMOL/L. NEITHER APAMIN NOR VARIATION IN EXTERNAL CA2+ CONCENTRATION AFFECTED THE NEGATIVE INOTROPIC EFFECTS OF THE k+ CHANNEL OPENERS. IT WAS SUGGESTED THAT THE MECHANICAL EFFECTS OF CROMAKALIM AND PINACIDIL ARE MEDIATED THROUGH THE ATP-SENSITIVE k+ CHANNELS IN THE HEART.

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Effects of Potassium Channel Blockers on the Negative Inotropic Responses Induced by Cromakalim and Pinacidil in Guinea Pig Atrium

Key Words
Cromakalim
Pinacidil
Tacrine
Glibenclamide
ATP-sensitive K^+ channel, cardiac
Atrial contraction
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Abstract
The K^+ channel openers cromakalim and pinacidil induced a concentration-dependent reduction in atrial contraction force with EC_{50} values of 25 ± 2 and 37 ± 2 μmol/l, respectively. This depressant effect was antagonised by 50 μmol/l tacrine which displaced the concentration-response curves of cromakalim and pinacidil to the right. The respective DR_{50} values were 3.8 and 2.3. Increasing the tacrine concentration (100 and 500 μmol/l) produced no additional effect on the concentration-response relationships. Addition of 1 μmol/l atropine enhanced the antagonism due to tacrine by increasing the DR_{50} value from 3.8 to 6.5 for cromakalim and from 2.3 to 5.2 for pinacidil. Glibenclamide, an ATP-sensitive K^+ channel blocker, competitively inhibited the negative inotropic effects of cromakalim and pinacidil. The respective dissociation constants for glibenclamide against cromakalim and pinacidil were 0.57 and 0.35 μmol/l. Neither apamin nor variation in external Ca^{2+} concentration affected the negative inotropic effects of the K^+ channel openers. It was suggested that the mechanical effects of cromakalim and pinacidil are mediated through the ATP-sensitive K^+ channels in the heart.

Introduction

Regulation of potassium channels in cardiac tissues is important in regulating normal heart functions, such as the control of cardiac contractility and the determination of cell membrane potential. Cromakalim (BRL 34915), a benzopyrane, and pinacidil, a cyanoguanidine, belong to a new class of antihypertensive agents [1, 2] which relax vascu-

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lar and cardiac smooth muscles by activation of K^+ channels [3, 4]. Increasing the efflux of K^+ results in the hyperpolarisation of the cell membranes, shortening of action potentials and reduction in the force of muscle contractions. These properties of cromakalim and pinacidil form the basis of their anti-hypertensive actions [5].

As there are many types of K^+ channels in the heart, all involved in the regulation of electrophysiological and contractile responses [6]; it will be of interest to find out the type of K^+ channels activated by cromakalim and pinacidil. We have previously shown that the centrally acting anti-cholinesterase, tacrine (THA), blocks the K^+ channels in the heart [7]. Glibenclamide, a sulphonylurea, was reported to antagonize cromakalim in guinea pig isolated trachealis muscle [8] and to block the ATP-dependent K^+ channel in insulin-secreting cells [9]. Studying the antagonism of the K^+ channel openers with tacrine and glibenclamide might provide information to characterise the K^+ channels activated by cromakalim and pinacidil in guinea pig atrium. In this study, the effects of potassium channel blockers on the atrial muscle relaxation induced by cromakalim and pinacidil were investigated. The results obtained are interpreted in relation to their activities at the K^+ channels.

Materials and Methods

Guinea Pig Atrial Preparations

Left atrial preparations were surgically removed from female guinea pigs weighing 250–400 g as described by Freeman and Turner [10]. Preparations were then placed in an organ bath containing heart Ringer's solution (composition mmol/l: NaCl 115, KCl 4.6, $CaCl_2$ 1.8, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 22, glucose 22, pH = 7.4) at 37 °C. The buffer was bubbled with carbogen (95%, O_2 and 5% CO_2) continuously. The preparations were allowed to equilibrate for 30 min before the experiment commenced. The atria were stimulated at 2 Hz and 10 V, and the tension of isometric contraction was recorded by a Shinkoh U-gauge (UL-2-120). The signal outputs were modulated by a Coulbourne transducer (S72-75) and recorded by a Graphtech linear recorder (WR-3071). Tension development was constant within the period of the experiments which lasted for approximately 3 h.

Data Analysis

The concentration-response curves were constructed by adding drugs cumulatively to the organ bath. Data are presented as means ± SEM and analysed according to the methods (ANOVA and Bonferroni) suggested by Wallenstein et al. [11].

Drugs

Cromakalim was donated by Beecham, UK, and pinacidil was a gift from Leo, Denmark. Tacrine was obtained from the Institute of Drug Technology, Melbourne, Australia. Apamin, atropine and glibenclamide were purchased from Sigma, St. Louis, Mo., USA.

Results

Effects of Cromakalim and Pinacidil on Atrial Contractility

Both cromakalim and pinacidil depressed the force of atrial contraction in a concentration-dependent manner. The effect of cromakalim was just perceptible at 10 µmol/l and rapidly reached a maximum suppression of approximately 80% of the control at 40 µmol/l. The effective concentration to reduce the force of contraction by 50% (EC_{50}) for cromakalim was 25 ± 2 µmol/l (fig. 1). Pinacidil showed a similar concentration-response relationship to cromakalim except that its potency was slightly weaker. This is indicated by the higher EC_{50} of 37 ± 2 µmol/l for pinacidil (fig. 1). There is however one noticeable difference between the negative inotropic effects of cromakalim and pinacidil. It took more than 30 min for the force of contraction to return to the control level after the

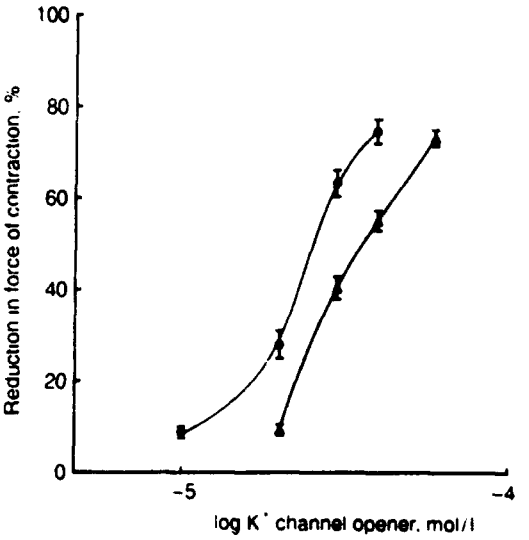

Fig. 1. The negative inotropic effects of cromakalim (●) and pinacidil (▲). Data are obtained from six experiments. Means ± SEM.

Table 1. Effects of varying concentrations of tacrine, and tacrine (100 µmol/l) plus atropine (1 µmol/l) on the negative inotropic responses induced by cromakalim and pinacidil

	Control	Tacrine			Tacrine (100 µmol/l) + atropine (1 µmol/l)
		50 µmol/l	100 µmol/l	500 µmol/l	
Pinacidil EC_{50}, µmol/l	36.8 ± 1.3	85.4 ± 3.1*	86.5 ± 4.2*	84.2 ± 0.5*	192.1 ± 2.2*,**
Cromakalim EC_{50}, µmol/l	27.5 ± 1.1	105.6 ± 2.6*	106.1 ± 2.7*	106.9 ± 1.0*	178.9 ± 1.7*,**

Each data point is calculated from five experiments and is expressed as mean ± SEM. Data are analysed by ANOVA and Bonferroni methods according to Wallenstein et al. [11]. * $p < 0.01$ vs. control, ** $p < 0.01$ vs. tacrine alone.

wash-out of pinacidil, but the depression was totally and rapidly reversed when cromakalim was removed.

Interaction with Tacrine

Tacrine at 50 µmol/l caused a parallel shift of the concentration-response curves of pinacidil and cromakalim to the right. The dose ratio at 50% reduction in the force of atrial contraction (DR_{50}) for cromakalim was 3.8, while that for pinacidil was 2.3. Increasing the concentration of tacrine to 100 and 500 µmol/l did not produce an additional effect on the concentration-response curves of cromakalim and pinacidil (table 1), suggesting the antagonism by tacrine is independent of its concentration.

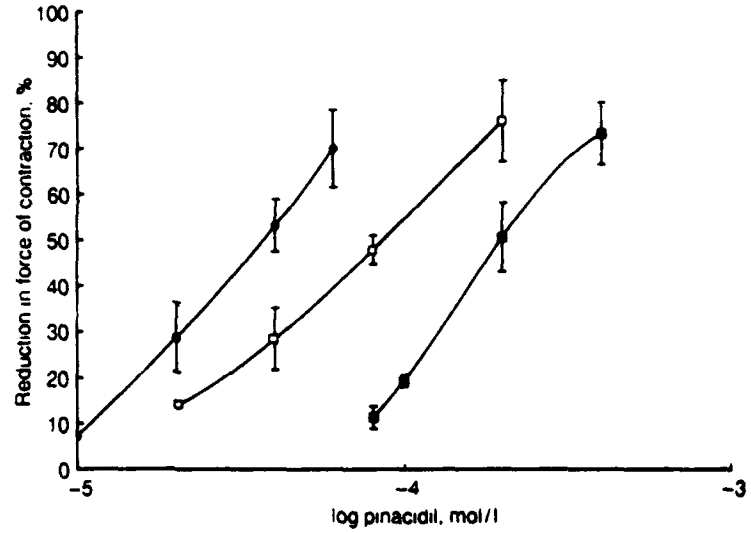

Fig. 2. The effects of 100 μmol/l tacrine (□), and 100 μmol/l tacrine + 1 μmol/l atropine (■) on the negative effect of pinacidil. ● = Controls. Data are obtained from five experiments. Means ± SEM.

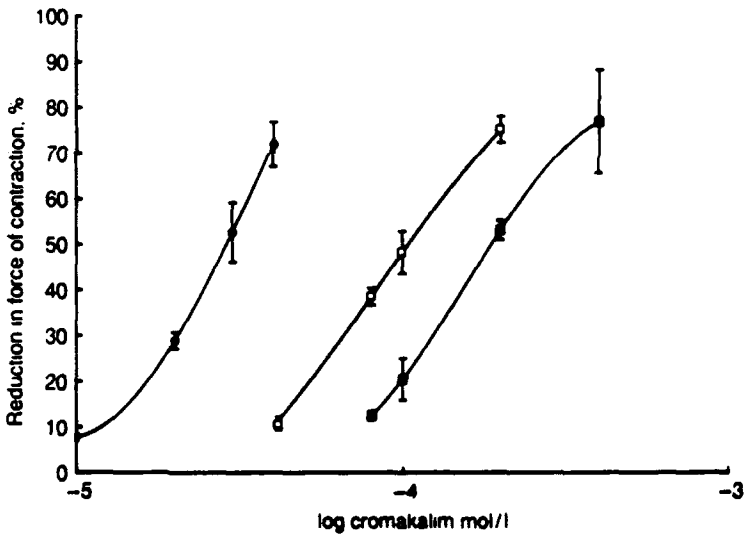

Fig. 3. The effects of 100 μmol/l tacrine (□); and 100 μmol/l tacrine + 1 μmol/l atropine (■) on the negative inotropic effect of cromakalim. ● = Controls. Data are obtained from five experiments. Means ± SEM.

Interaction with Tacrine and Atropine

To investigate if the cholinergic effects of tacrine are involved in antagonising the depressant effects induced by cromakalim and pinacidil, their concentration-response relationships were studied in the presence of 100 μmol/l tacrine and 1 μmol/l atropine, an anti-muscarinic agent. The concentration-response curves for cromakalim and pinacidil were both shifted further to the right (fig. 2, 3). The DR_{50} value was increased from 3.8 to 6.5 for cromakalim and from 2.3 to 5.2 for pinacidil.

Interactions with Glibenclamide

Glibenclamide itself produced a concentration-dependent reduction in atrial force of contraction. The effect was just perceptible at

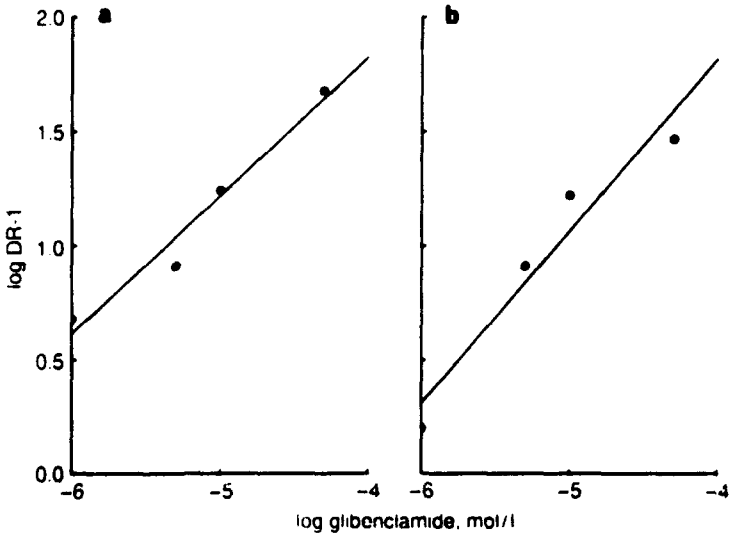

Fig. 4. Schild plot of the antagonism of the negative inotropic effect of pinacidil (**a**) and cromakalim (**b**) by glibenclamide. Data are obtained from thirteen (**a**) and twelve experiments (**b**). Means ± SEM. DR = Dose ratio.

1 μmol/l which reduced the force of the control by 8% and reached the maximum at 20 μmol/l with a reduction of 55%. The negative inotropic effects of cromakalim and pinacidil were both antagonised by glibenclamide by displacing their concentration-response curves to the right in a concentration-dependent manner (1–50 μmol/l). Analysis of the data with Schild plot [12] showed a linear relationship between the (dose ratio-1) and glibenclamide concentrations against the two potassium channel openers (fig. 4). The slope of the plot for cromakalim was 0.90 and for pinacidil was 0.88 which were not significantly different from unity ($p < 0.05$). The apparent dissociation constant (K_a) for glibenclamide was 0.57 μmol/l against cromakalim and 0.35 μmol/l against pinacidil. These comparable dissociation constants suggested that they may be acting at a common site.

Interactions with Apamin and Exogenous Ca^{2+}

Apamin (10–100 nmol/l) had no effect on the depressant effects of cromakalim and pinacidil. Similarly, reducing the Ca^{2+} content to one third of the original strength of the heart Ringer's solution or increasing it by 50% produced no effect on the negative inotropism induced by the two K^+ channel openers.

Discussion

Activation of the K^+ channels in cardiac sarcolemma by cromakalim and pinacidil has been suggested to cause a shortening of action potential, reducing the time for Ca^{2+} influx and thus resulting in a reduction in the contraction force [13]. Our results for cromakalim and pinacidil agreed with this proposal. Both of them, even at higher concentrations, could not completely abolish atrial contraction. This is different to the suppression caused by diltiazem and nifedipine, both of which are Ca^{2+} channel blockers and are capa-

ble of totally relaxing atrial muscle [14]. The residual contraction of the atrium in the presence of high concentrations of cromakalim and pinacidil is thus an indication that these K^+ channel openers do not directly affect the inward Ca^{2+} current. The potency of the relaxing properties of cromakalim and pinacidil in guinea pig atrium is much weaker than that reported in canine atrial muscle [15], in guinea pig trachea [16, 17], in rabbit arteries [18] and in human skeletal muscle fibres [19]. It is not known whether the difference in potency is linked to differences in channel properties between different tissues or species.

Tacrine antagonized the cromakalim- and pinacidil-induced negative inotropic effects in a concentration-independent manner (table 1). This effect is apparently not arisen from a simple competitive antagonism at a site common to tacrine, cromakalim and pinacidil. Tacrine was found to block the inwardly rectifying K^+ current in guinea pig isolated ventricular myocytes [20] and inhibit the slow outward K^+ current in the peptidergic neurons of snail [21]. If these are the primary action sites for tacrine, our results suggest that cromakalim and pinacidil do not activate these K^+ channels. The uncompetitive antagonism observed is probably a functional rather than a specific one. Tacrine has been reported to inhibit acetylcholinesterase and to block muscarinic acetylcholine (M-ACh) receptors [7, 22]. Inhibition of the cholinesterase by sub-micromolar concentration of tacrine (K_i = 1.8 μmol/l) would result in a transient accumulation of acetylcholine and increase the frequency of M-ACh receptor activation. This would lead to an increase in K^+ efflux, thereby opposing the effects of K^+ channel blockade due to tacrine. However, at 100 μmol/l tacrine, all M-ACh receptors would have been blocked. Thus the enhancement of tacrine by atropine to antagonise the negative inotropic effects of the two K^+ channel openers is therefore inexplicable in the context of M-ACh receptor blockade.

Atropine is a potent muscarinic antagonist, with an affinity of 2×10^9 l/mol for M-ACh receptors [23]. It may be possible that excessive atropine (1 μmol/l) used in this study displaced tacrine at the M-ACh sites, releasing free tacrine to block the K^+ channels. Additional work is required to clarify this point.

Glibenclamide had been found to block ATP-sensitive K^+ channels in insulin-secreting cells [9], pancreatic β-cells [24] and cardiac muscles [25]. It was also reported that glibenclamide antagonised competitively with cromakalim and pinacidil in vascular tissues and airway smooth muscles [26, 27]. Our results demonstrated that glibenclamide antagonised the negative inotropic effects of cromakalim and pinacidil on guinea pig atrium. The Schild plots give a slope of 0.90 for cromakalim and 0.88 for pinacidil suggesting the antagonism is of the simple competitive type. The apparent dissociation constants for glibenclamide to act against cromakalim (0.57 μmol/l) and pinacidil (0.35 μmol/l) agree well with the values reported in other studies [27, 28]. It is also noted that the values of the two constants are comparable to each other, hence suggesting that cromakalim, pinacidil and glibenclamide could interact at a common site. This site is most likely the atrial ATP-sensitive K^+ channels which are also the primary site for glibenclamide [25]. Further experiments in the presence of apamin, which blocks small conductance Ca^{2+}-activated K^+ channels [29], or by varying the concentration of exogenous Ca^{2+} produced no changes in the muscle relaxation by cromakalim and pinacidil. It appears that cromakalim and pinacidil do not open Ca^{2+}-activated K^+ channels.

The current study demonstrated that the mechanical effects of cromakalim and pinacidil were inhibited competitively by glibenclamide; uncompetitively by tacrine and were unaffected by apamin or by varying the exogenous Ca^{2+} contents. The excitation-contraction coupling in atrial muscles has not been fully understood. If the mechanical effects of cromakalim and pinacidil are primarily associated with their potassium channel opening properties, it can be established that they mediate the depression through opening of the ATP-sensitive K^+ channels.

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